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Vector for the Secretion of Western Equine Encephalitis Antigen

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REPORT FOR PROJECT ENTITLED

VECTOR FOR THE SECRETION OF

WESTERN EQUINE ENCEPHALITIS ANTIGEN.

CONTRACT NO. W 7702-0-P080

(1 JULY 2000 - 20 OCTOBER 2000)

SUBMITTED TO: DR. L. NAGATA
CHEMICAL BIOLOGICAL DEFENCE SECTION
DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
P.O. BOX 4000, STATION MAIN
MEDICINE HAT, ALBERTA
T1A 8K6

SUBMITTED BY: DR. P.L. MONETTE, PRESIDENT
ARBUTUS LIFE SCIENCES CORPORATION

OCTOBER 20, 2000

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INTRODUCTION

Western Equine Encephalitis (WEE) virus is a member of the *Alphavirus* genus in the family *Togaviridae* (1). The virus genome consists of (+)sense single-stranded RNA encapsidated within an enveloped virion vectored by mosquitoes. The virus can cause encephalitis and central nervous system (CNS) disorders in horses, humans, birds and other species (2-5). The fatality rate in humans, 3-7 %, is lower than that of the related alphavirus Eastern Equine Encephalitis (EEE) virus, but infections with WEE virus are sufficiently debilitating that the virus constitutes a potential biological warfare agent.

Currently, immunodetection and immunotherapy offer the best protection against WEE virus, EEE virus and other biological warfare agents. Monoclonal antibody technology and the cloning of recombinant antibodies represent powerful tools for the detection and identification of alphaviruses, as well as for prophylaxis and treatment of alphavirus infections. In order to derive the maximum potential benefit from these approaches, a safe and reliable source of antigen is required for the characterization of antibodies.

The conventional method for producing viral antigen consists of growing and purifying the virus, inactivating it, and then safety testing the product. Safety issues are of great concern when using this procedure, particularly when working with viruses requiring biological level 3 containment. A further disadvantage is that the formaldehyde or irradiation used to inactivate the virus may damage the antigenic epitopes.

As an alternative to virus purification, it is now possible to express selected virus genes in prokaryotic or eukaryotic systems. The latter are preferred, as they often allow post-translational modification of the foreign protein. The baculovirus insect cell expression system is a widely utilized system allowing high level production of foreign proteins. The disadvantage of this system is that a recombinant baculovirus must be constructed, maintained and used to infect cells each time protein is needed. Recent technology has led to the development of insect expression vectors which can be grown and manipulated in bacteria, and then introduced directly into insect cells. The pIZT/V5-His InsectSelect™ Expression Vector from Invitrogen Corporation possesses these attributes (6). It can be used for the stable expression of heterologous proteins in lepidopteran

insect cell lines. With this technology, it is possible to produce WEE capsid protein, free of any infectious nucleic acid. The translation product of a construct consisting of the pIZT/V5-His vector containing a WEE structural gene insert provides a safe and authentic source of antigen for the characterization of antibodies, and may prove useful as a subunit vaccine for alphaviruses.

The assembly of viral capsid proteins into a virion generates a number of epitopes which do not exist in the capsid protein subunit itself. These epitopes are of great interest, as they occur on the surface of infectious particles, and are thus potential targets for the host immune response. The assembly of capsid proteins into a viral capsid is partly controlled by a virus genome-specific 'packaging signal' (1). The WEE packaging signal has been identified. It is thus possible to engineer a recombinant WEE expression vector which contains this signal. Expression of such a vector in insect cells may lead to the production of noninfectious particles of WEE virus.

OBJECTIVE AND WORK OUTLINE

The objective of this project is to engineer a recombinant WEE expression vector which contains a WEE packaging signal, and which can be used in insect cells. The sub-objectives to be addressed in achieving this goal are as follow:

1. preparation of WEE packaging signal for ligation into vector
2. preparation of vector
3. preparation of construct and transformation of bacterial host cells
4. screening of transformants
5. transfection of insect cells

RESULTS

1. Preparation (annealing) of packaging signal.

The SA designed a linker to go into *Xba* I and *Sac* II sites of the pIZT-WEE insect vector previously constructed (Contract No. W7702-9-P066). A *Pvu* II site was inserted into the linker for screening. The SA provided the linker as two single-stranded DNA segments, Oligo 8 WEE (+) and Oligo 9 WEE (-) (Fig. 1).

Oligo 8 WEE (+)

5' **CTAG**ACaGCTGCGCGGCGAGAGGGTTTCTTTTGCTGTGTGTACGT**GC** 3'

3' TGtCGACGCGCCGCTCTCCCAAAGAAAACGACACACATGCA 5'

Oligo 9 WEE (-)

Figure 1. Oligo 8 WEE (+) [top] and Oligo 9 WEE (-) [bottom], ssDNA segments which anneal into a linker for the WEE packaging signal.

These were each dissolved in 1 mL water and their concentrations were determined. Oligo 8 and 9 had concentrations of 911 and 787 μg/mL, respectively. For annealing, 10 μL of each ssDNA was added to 480 μL water. The concentrations of Oligo 8 and 9 DNA in the annealing mixture were thus 18 and 16 μg/mL, respectively. The mixture was heated at 75 °C for 10 min and then allowed to cool slowly to room temperature.

2. Preparation (restriction enzyme digestion) of vector.

The previously constructed pIZT-WEE insect vector was resuspended in water to a concentration of 1 μg/mL. Two μg plasmid DNA was then digested at 37 °C for 2 h with 40 units each of *Xba* I and *Sac* II (7; New England BioLabs, Mississauga, ON). Enzymes were then inactivated by incubation at 65 °C for 20 min.

3. Preparation of construct and transformation of E. coli.

The restriction enzyme-digested pIZT-WEE vector (0.06 μg) was mixed with an excess of annealed linkers (2.7 μg). The mixture was incubated at 16 °C overnight in the presence of 1 unit of T4 DNA ligase

(New England BioLabs). *E. coli* DH5α competent cells (Gibco/BRL, Burlington, ON) were then transformed with the ligation products (7).

4. Screening of transformants.

Transformants were selected on Low Salt LB plates (1.0 % tryptone, 0.5 % yeast extract, 0.5 % NaCl, pH 7.5), containing 50 μg/mL Zeocin, as per Invitrogen recommendations (6). A 5 mL culture of each selected transformant was prepared. The plasmid DNA from these cultures was isolated and digested with either *Sac* II or *Pvu* II (7; New England BioLabs). Restriction digests were analyzed on 1.2 % agarose gels. A transformant, designated number 12, was selected for further work on the basis that its plasmid DNA was not digested by *Sac* II, but was susceptible to *Pvu* II (Fig. 2).

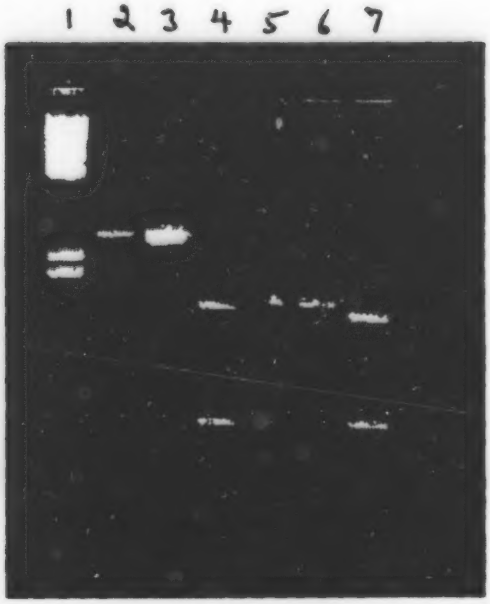

Figure 2. Agarose gel electrophoretic analysis of restriction enzyme-digested plasmid preparations from selected transformants. Lane 1 = Lambda DNA/*Hind* III markers (Promega, Madison, WI). The sizes of the fragments (top to bottom) are 23,130 bp, 9,416 bp, 6,557 bp, 4,361 bp, 2,322 bp and 2,027 bp. Lane 2 = undigested plasmid DNA from transformant number 11. Lane 3 = *Sac* II-digested plasmid DNA from number 11. Lane 4 = *Pvu* II-digested plasmid DNA from number 11. Lane 5 = undigested plasmid DNA from transformant number 12. Lane 6 = *Sac* II-digested plasmid DNA from number 12. Lane 7 = *Pvu* II-digested DNA from number 12.

5. Transfection of insect cells.

Plasmid DNA was purified from a 40 mL overnight culture of DH5α transformant number 12. High Five cells were transfected with this DNA, and a stable cell line was selected with Zeocin (6).

DISCUSSION

The expression of foreign genes in insect cell lines allows for post-translational modifications of the expressed proteins, a process which does not necessarily occur when the genes of interest are expressed in prokaryotic host cells. Systems have recently been developed whereby lepidopteran cell lines can be transformed with vectors which then provide for the stable and high-level expression of foreign genes. These systems represent a substantial improvement over the baculovirus insect cell expression system.

The objective of this project was to engineer a recombinant WEE expression vector which contains a WEE packaging signal, and which can be used in insect cells. A linker with the packaging signal was designed and provided by the SA. This linker was incorporated into a previously prepared pIZT-WEE vector. *E. coli* DH5a were transformed with the new vector and screened by restriction enzyme analysis of their plasmid DNA. A transformant was selected on the basis that its plasmid DNA was not susceptible to digestion by *Sac* II, but was susceptible to digestion by *Pvu* II. Plasmid DNA was purified from a large-scale preparation of this transformant, and used to transfect High Five cells. A stable cell line was selected with Zeocin.

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